Central histamine contributed to temperature-induced polypnea in mice

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Centralhistaminergicneuronshaveawidedistribution
in the brain (10, 20) and affect thermoregulation,
feeding, circadian rhythms, and various autonomic
functions such as cardiovascular functions (22). Central
histamine causes hyperthermia in mice (2, 23) and
activates the heat loss mechanisms in behavioral studies
(7, 8). Considering the thermoregulatory effect and
the wide distribution, central histamine may influence
the respiratory system for heat loss. Recently, several
studies show that central histamine decreases tracheal
tension (11) and that histamine release is autoregu-
lated by H3 receptors in the medulla oblongata in
rabbits (12). As yet, however, little is known about
histamine’s effect on breathing pattern.

In this study, we first made a body plethysmograph
for a conscious mouse and measured respiratory vari-
ables at two different body temperatures to clarify the
effect of body temperature on breathing pattern. Then
we investigated whether central histamine affects
breathing pattern with the administration of S(+)-α-
fluoromethylhistidine hydrochloride (α-FMH; Re-
search Biochemicals International, Natick, MA), which
is a specific inhibitor of histidine decarboxylase (5) and
causes a marked decrease in histidine decarboxylase
activity and a concomitant decrease in neural hista-
mine (13). Breathing pattern was characterized with
the relationships between tidal volume (VT) and in-
spiratory time (TI) and expiratory time (TE) obtained
by increasing hypercapnia.

The purpose of this study was to investigate how
central histamine contributes to the effect of body
temperature on breathing pattern in conscious mice.
Therefore, we examined 1) the effect of temperature on
breathing pattern in mice and 2) whether central histi-
amine influences breathing pattern. Finally, the rela-
tionship between the effect of temperature and central
histamine on breathing pattern was discussed.

MATERIALS AND METHODS

Animals. Inbred male C57BL/6N mice (8 wk old) used in
this experiment were provided with food and water ad libi-
tum, housed at a controlled temperature (22 ± 1°C), and

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exposed to a daily 12:12-h light-dark cycle; all experiments were conducted in an environmentally controlled room at a temperature of 22 ± 1°C.

Measurement of lung ventilation. Ventilation was measured by double-chamber plethysmography as illustrated in Fig. 1. We made this system by referring to Vijayaraghavan et al. (30). A continuous airflow through the chambers was produced by a vacuum pump at a flow rate of 150 ml/min through a critical orifice. Airflow of the head chamber was measured by a pneumotachograph (TV-241T and TP-602T, Nihon Kohden) through the addition bias flow and was recorded on an analog tape recorder (TEAC, R-71). The data stored in the tapes were fed into analysis systems (MacLab, ADInstruments) on a Power PC (Macintosh, Apple), at a sampling rate of 10,000 Hz. A total of 10 consecutive breaths was analyzed under each condition in a steady state. Ti (s), Tt (s), total breath duration (Tr, s), and VT (ml BTPS) were measured for each breath and averaged; f (breaths/min) was determined as 60/Tr. VT was calibrated by injecting 0.5 ml of ambient air (22 ± 1°C) with a syringe through a small hole of the head chamber, which was sealed during other procedures. An elevation of the temperature of the head chamber was practically avoided by the continuous bias flow through the experiments. VT was calculated using the following equation

\[
VT = \left(273 + T_b\right)/\left(273 + T_{am}\right) \times \left(760 - P_{amH_2O}\right)/\left(760 - P_{H_2O}\right) - P_{H_2O} \times 0.5/V_{cal} \times VT_{ATPS}
\]

where T_b is rectal temperature (°C); T_{am} is ambient temperature (°C); P_{amH_2O} and P_{H_2O} are the water vapor pressures (mmHg) in the ambient air and the alveoli, respectively; and VT_{ATPS} is VT at ATPS without calibration (ml). The injected volume into the head chamber was 0.5 (ml ATPS) for calibration, recorded as V_{cal} (ml ATPS) on the PC. Minute ventilation (V_{E}, ml BTPS) was determined as f × VT. VT and V_{E} were normalized by body weight per 10 g.

Hypercapnic protocol. Mice were anesthetized with pentobarbital sodium (12.5 mg/kg ip) for setting in the plethysmograph. Each mouse was acclimatized to the chambers for >60 min before the assessment of ventilatory function. After the mouse recovered from anesthesia and momentary hypothermia caused by pentobarbital sodium, a bag (8 liters) was connected to the head chamber via its inlet port. Three bags

![Fig. 1. Ventilation was measured by double-chamber plethysmography. AD converter, analog-to-digital converter. We made this system by referring to Vijayaraghavan et al. (30).](http://jap.physiology.org/)

![Fig. 2. Comparison of respiratory frequency (top) responses to inspired CO2 at 36–37°C (●) and at 39°C (○), tidal volume (VT, middle), and minute ventilation (bottom). Significant main effect for inspired CO2 (***P < 0.001); significant main effect for temperature († † † † P < 0.001).](http://jap.physiology.org/)
contained different hypercapnic gas mixtures (5, 7, and 9% CO2 in O2). Stepwise changes in the inspired gas were produced by quickly switching to a bag containing a different gas mixture. The CO2 mixtures were applied for 4–6 min until a new steady state had been reached and were then changed to the next step. Parameters were measured at the end of each period. Rectal temperature was continuously monitored by means of a thermistor inserted into the rectum and controlled at the target temperature by a heating light from outside the chamber with an animal blanket controller (ATB-1100, Nihon Kohden).

Effects of body temperature. The experiments were performed on a total of 11 mice. Mice were loaded with hypercapnic gas mixtures according to the protocol at a body temperature of 36–37°C. After this examination, mice were allowed to rest for 3 days in preparation for the next examination. At the next examination, the body temperature was gradually raised with the controller to 39°C over a period of 30 min; subsequently, the hypercapnic protocol was run in a similar way.

Effects of α-FMH. We investigated the effect of α-FMH on breathing patterns at two different temperatures to define the role of central histamine. First, we carried out the procedure under a normal temperature (~37°C) using 14 mice divided into two groups of 7. α-FMH was dissolved in saline and injected at a dose of 100 mg/kg ip, and the same volume of saline (0.2 ml) was given to the control animals. This dosage of α-FMH depletes neural histamine in mice in 4 h (13). All respiratory assessments were carried out 4 h after the injection; after a 3-h period, the mice were anesthetized and positioned in the plethysmograph, and each mouse was then placed in the chambers for an additional 1 h and was subsequently loaded with the hypercapnic gas mixture. To observe the effect of α-FMH under the raised temperature, we conducted the same procedure at a body temperature of 39°C with the controller, using another group of 12 mice divided into two groups of 6.

Statistical analysis. A commercially available software package (SPSS, SPSS Japan) was employed. Data were analyzed with two-way ANOVA to test for effects of CO2 and temperature or CO2 and α-FMH. Breathing pattern was statistically evaluated by the slopes and the extrapolated VT of linear regression analysis between VT and TI or VT and TE for each mouse. The mean slope and the VT of the VT-TI or VT-TE line for each condition was obtained from the average of each mouse. The difference in the slopes between groups was examined by t-test. Unless a difference in the slopes was detected, the difference in VT was consequently examined by the t-test at two points, the earliest and the latest time of overlap between the two lines. If an overlap did not exist, the t-test was performed at the midpoint of time between two lines. Statistical significance was accepted for $P < 0.05$. Results were expressed as means ± SE.

RESULTS

Effects of body temperature. Figure 2 shows the effects of body temperature on the response of $f$, $V_T$, and
$\dot{V}E$ to hypercapnia. Responses to inspired CO$_2$ on these variables were evaluated in 11 mice at 36–37°C and at 39°C. For all variables, there were significant effects of CO$_2$ inhalation (all $P < 0.001$); CO$_2$ produced significant increases in $f$, $V_T$, and $V\dot{E}$ at both temperatures. There was a significant increase in $f$ ($P < 0.001$) and a significant decrease in $V_T$ ($P < 0.05$) at 39°C. Consequently, changes in body temperature had no effect on $V\dot{E}$. Because there was no significant interaction between CO$_2$ and temperature according to ANOVA, the responsiveness of $f$, $V_T$, and $V\dot{E}$ to inspired CO$_2$ was not changed by temperature.

Figure 3 shows the effects of CO$_2$ and temperature on $Ti$ and $Te$. There were significant decreases in both $Ti$ and $Te$ (all $P < 0.001$), which reflected the increase in $f$. No interactions between CO$_2$ and temperature existed.

Figure 4 shows the mean $V_T$-$Ti$ and $V_T$-$Te$ relationships during the stepwise CO$_2$ inhalation at the two different body temperatures. Linear regression lines were obtained for each mouse (all $P < 0.05$). CO$_2$ inhalation increased $V_T$ with a reduction in $Ti$ and $Te$. There was no significant difference between the slopes of the regression lines of $V_T$-$Ti$ ($-2.007 \pm 0.316$ ml/s at 36–37°C and $-2.341 \pm 0.410$ ml/s at 39°C). $V_T$, however, significantly decreased from $0.161 \pm 0.006$ to $0.126 \pm 0.008$ ml at the same level of $Ti$ of 0.11 s at 39°C ($P < 0.01$). Thus the increase in body temperature shifted the $V_T$-$Ti$ line below to the left. On the other hand, the slope of the $V_T$-$Te$ line became steeper from $-0.706 \pm 0.142$ to $-1.377 \pm 0.256$ ml/s and moved to the left with the body temperature raised. In summary, the raised body temperature shifted both the $V_T$-$Ti$ and the $V_T$-$Te$ lines to the left.

Effects of $\alpha$-FMH. The effects of $\alpha$-FMH on responses of $f$, $V_T$, and $V\dot{E}$ to hypercapnia at 37°C and 39°C are shown in Fig. 5, A and B, respectively. CO$_2$ significantly increased $f$, $V_T$, and $V\dot{E}$ (all $P < 0.001$). There were no differences between the groups in $f$ or $V_T$ nor $V\dot{E}$ at 37°C, whereas, at 39°C, $f$ in the $\alpha$-FMH-treated group was lower than in the vehicle-treated group ($P < 0.05$).

![Fig. 5. Comparison of respiratory frequency responses (top) to inspired CO$_2$ with saline (○) and with $\alpha$-FMH (●), $V_T$ (middle), and minute ventilation (bottom) at 36–37°C (A) and 39°C (B). Significant main effect for inspired CO$_2$ (***,$P < 0.001$); significant main effect for $\alpha$-FMH (†,$P < 0.05$).](http://jap.physiology.org/DownloadedFrom)
0.05). On the other hand, neither VT nor VE was affected by α-FMH. According to ANOVA, there were no significant interactions between the effects of CO2 and α-FMH.

The effects of α-FMH on TI and TE at 37°C and 39°C are shown in Fig. 6A and B, respectively. There were no differences between the groups in TI or TE at 37°C. In contrast, α-FMH significantly prolonged TE (P < 0.05) but had no effect on TI at 39°C. The reduction in f by α-FMH at 39°C, therefore, was caused by prolonged TE.

Figure 7 shows the mean relationships of VT-TI and VT-TE during CO2 inhalation. The relationships of VT-TI and VT-TE were fitted by linear regression lines (all P < 0.05). There were no differences between vehicle- and α-FMH-treated groups in the relationships of VT-TI at both temperatures. On the other hand, the slope of VT-TE was more gentle in the α-FMH-treated group (−1.421 ± 0.355 ml/s) than in the vehicle-treated group (−1.311 ± 0.667 ml/s) (P < 0.05) at 39°C; α-FMH shifted the VT-TE relationship to the right. Unlike the results at 39°C, α-FMH did not change VT-TE at 37°C. In summary, breathing pattern was influenced by α-FMH at the raised body temperature but not at the normal temperature.

DISCUSSION

In the present study, we report a novel role for central histamine in altering breathing pattern in conscious mice. First, we have demonstrated that a raised body temperature caused an increase in f with reductions in TI and TE in conscious mice. In addition, we have shown, by means of results obtained from experiments using α-FMH, that central histamine contributed to temperature-induced polypnea with a reduction in TE.

Effects of body temperature. This study showed that the raised body temperature increased f with reductions of both TI and TE but did not change VE in conscious mice. These results were essentially in agreement with previous studies in other species (3, 19, 27, 28). Other research has also studied the relationship between VE and either inspired, alveolar, or arterial levels of CO2. When body temperature is raised, VE response to CO2 is increased, mainly because of the augmentation in f (21, 25, 30, 31). These approaches, however, have often adopted animal models under anesthesia. Because anesthetics have an effect on the CO2 response (6), the application of these results to the conscious model needs careful consideration. On the other hand, in another study using a conscious animal model, the inspired CO2 response was elevated slightly in hyperthermia (17). However, we were unable to measure alveolar CO2 in conscious mice because the possibility of the animal suffering from tracheotomy prevented us from using a gas analyzer. An inconsistency between results might come partly from this experimental limitation in conscious models.

To advance our understanding, we attempted to clarify the effect of temperature with analyses of the relationships between VT and TI and VT and TE. The
relationship between VT and TI was previously described by Clark and von Euler (3). In our study, the raised body temperature allowed the VT-TI line to shift below to the left. According to this result, VT at the raised temperatures was lower than at the normal temperature. A similar effect is reported in anesthetized cats (1). On the contrary, other studies demonstrate that indeed a raised body temperature shortens TI but does not alter phrenic tidal amplitude at the same alveolar CO2 level in anesthetized cats (27, 29). Unlike the results under anesthesia, in a conscious and spontaneous breathing condition, mice were able to adjust their VT so that hyperventilation caused by the continuous raised temperature would not proceed excessively. Therefore, a plausible explanation for the reduction in VT observed in our study was that an excess of ventilation evoked by a continuous raised temperature lowered the threshold for the inspiratory off switch because of a reduction of CO2 in the body.

Lung ventilation represents the major route of CO2 emission as well as an important pathway of heat loss. The metabolic rate modifies the breathing pattern to match the needs. In addition, raised body temperatures are accompanied by changes in metabolic rate (18). The metabolic effect, which is mediated by CO2, could account for all of the alteration in breathing pattern. However, changes in CO2 do not shift the VT-TI line and simply move VT and TI on the same line (1). Therefore, the metabolic effect failed to explain the whole shift of the VT-TI line. In addition, VT-TE was also expressed as a line at the same body temperature with increasing hypercapnia and was shifted wholly to the left by the raised temperature in our study. Therefore, the whole shifts of the VT-TI and VT-TE suggest that pathways different from the metabolic effect also contribute to changes in breathing patterns.

**Effects of α-FMH.** In this study, we examined the effect of central histamine on breathing patterns by using α-FMH. We found that central histamine contributed to temperature-induced polypnea in mice.

At the raised body temperature, f was lower with the prolongation of Te in α-FMH-treated mice, whereas at the normal temperature there was no difference in f between groups. These results indicate that the effect of central histamine increased f with a reduction in Te at a raised body temperature. The VT-Ti and the VT-TE analyses supported this; α-FMH shifted VT-TE to the right but not VT-Ti at the raised temperature, whereas neither VT-Ti nor VT-TE was affected by α-FMH at the normal temperature.

The fact that central histamine affects not Ti but Te also indicates that each parameter is able to change independently. The control theory of rate and depth proposed by Clark and von Euler (3), which is ex-

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**Fig. 7.** α-FMH resulted in no differences between slopes or VT at the same levels of time in VT-Ti (left) or VT-TE (right) at 36–37°C (A), whereas α-FMH changed the slopes of VT-TE (*P < 0.05) at 39°C (B), but VT-Ti did not change at 39°C. Solid lines, regression lines with saline; dotted lines, regression lines with α-FMH; Ss, slopes with saline; Sf, slopes with α-FMH.
plained by off-switch mechanisms located in the lower brain stem, cannot explain this fact, because Te is therein basically dependent on Ti. To the contrary, another study suggests that there is a separate control of Ti and Te, and increases in f are associated with reductions in Te rather than Ti in a conscious state (15); in addition, in a conscious human study, the higher neural center is suggested to affect predominantly f, especially Te (16). Therefore, these results suggest that the effects of higher neural centers, including central histamine, change Te independently in a conscious condition.

Findings of thermoregulation by central histamine have accumulated. Histamine immunoreactive neural fibers are distributed in the preoptic area known as the thermoregulatory center (10, 20). Histamine, the content of which in the hypothalamus increases at a high environmental temperature (4), activates the warm sensitive neurons in the preoptic area (24). Although the connection between the thermoregulatory and the respiratory centers has not been clearly defined, central histamine may act on breathing patterns via thermoregulatory pathways.

In general, changes in lung mechanics affect breathing patterns with inputs from pulmonary stretch receptors. Although peripheral administrations of histamine have little effect on lung mechanics in mice (9, 14), an administration of histamine into the fourth rami of the cervical sympathetic nerve (2) sensitized to affect predominantly f, especially Te (16).

In conclusion, we have characterized the effect of histamine on breathing pattern changes in our findings.

In conclusion, we have characterized the effect of central histamine on breathing pattern. Central histamine contributed to an increase in f caused by a raised body temperature with a reduction in Te and formed a part of the temperature effect in conscious mice.

REFERENCES


